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We use this protocol and it's working

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Nucleic acids extraction from single cell using MasterPure Complete DNA purification (Epicenter) V.2

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ABSTRACT

Radiolaria are protists which can't be cultivated. These microorganisms have to be isolated by single-cell for genetic identification and it can be difficult to get clean DNA.

Here we optimized a DNA extraction protocol from protist single-cell.

It works very well on single-cell Radiolaria, Foraminifers, but also Ciliates, Dinoflagellates, Diatoms.

MATERIALS

Material and Equipment :

- Glass bent micropipettes.
- Silicone Tubing (Cole-Parmer, Tube silicone platinum LS T17 ref. [FV-96410-17](#))
- StereoMicroscope or binocular microscope.
- Petridishes Diameter 70mm.
- Centrifuge 5417R (Eppendorf)
- Thermomixer Eppendorf

Kit, Reagents and Chemicals :

- **MasterPure Complete DNA and RNA purification kit** (Epicenter Illumina, ref MC85200)
Enzymes (Proteinase K, RNase, DNase) are stored at -20°C
Other reagents from the kit are stored at room temperature.
- Ethanol absolute, molecular biology quality.
- Propan-2-ol quality molecular biology.
- 0,22µm filtered Seawater or artificial Seawater.
- Ice for enzyme storage during labwork.

Wear labcoat, gloves.
Decontaminate all the surface area, and equipments (rotor, racks, pipettes...) with Ethanol 70% and DNA away.
Works with filter tips.

BEFORE START INSTRUCTIONS

Prepare Ethanol 70% from absolute Ethanol : Mix 35 mL of absolute Ethanol with 15 mL nuclease-free water in a Falcon 50mL Store at -20°C.

1. Cell Isolation

- 1 Isolate individually protist cells (at least 50µm in length) using a glass bent micropipette under a binocular microscope.
- 2 Wash each cell in three successive baths of 0.22µm-filtered and sterile seawater.
- 3 Transfer subsequently cells in a 1.5mL sterile microtube.
- 4 Add 30 µL of lysis buffer (Tissue and Cell Lysis Solution from MasterPure™ DNA and RNA Purification Kit, Epicenter) and store at -20°C.

2. Cell lysis

- 5 Pellet cells by centrifugation (2 min at Vmax), throw the supernatant, let ~25-30 µL of liquid.

- 6 Dilute 1 μL of Proteinase K in 300 μL de lysis solution Tissue et Cellule for each sample. Vortex 10 sec for resuspending cells (facultative).
- 7 Add 300 μL of mix Proteinase K + lysis solution Tissue et Cellule in each sample. Vortex.
- 8 Incubate 15 min at 65°C , 1000 rpm. Put samples in ice 3-5 min.

3. Total nucleic acids precipitation

- 9 Add 150 μL MPC reagent to 300 μL of lysed sample. Vortex.

4-+ 500 μL Propan-2-ol. Mix per inversion.
5-
6
7-+ 500 μL d'EtOH 70%. Don't vortex, mix gently the support. Spin 5min at V_{max} ;at 12°C .
8-Discard a maximum of supernatant with precaution, without touching the pellet.
9-Let dry 5-10 min at RT.
10-
10 Spin 10 min at 11 000 g, 12°C . If there is no pellet, add more 25 μL MPC buffer and spin again 10 min at 11 000 g, 12°C .

11 Transfer the supernatant ion a new clean microtube (1,5 mL), discard the pellet.
(To keep the squeletton, keep the tube with the pellet, add 500 μL MilliQ Water and store at - 20°C).

12 Add 500 μL of Isopropanol. Mix per inversion. Spin 10min at V_{max} , 12°C .

- 13 Discard the supernatant with precaution, without touching the pellet.
- 14 Add 500 μ L of Ethanol 70%. Don't vortex, mix gently the support. Spin 5min at Vmax ;at 12°C.
- 15 Discard a maximum of supernatant with precaution, without touching the pellet.
- 16 Let dry 5-10 min at room temperature..
- 17 Elute in 25 μ L of TE1x buffer.
Vortex and spin shortly.
Store at - 80°C.